Selective elimination of photodamaged chloroplasts by autophagy: intracellular process and induction mechanism

博士論文 (要約)

Selective elimination of photodamaged chloroplasts by autophagy: intracellular process and induction mechanism

光障害葉緑体を除去する選択的オートファジー経路とその誘導機構の解析

平成 30 年度

東北大学大学院生命科学研究科
生態システム生命科学専攻

中村　咲耶

Selective elimination of photodamaged chloroplasts by autophagy: intracellular process and induction mechanism

（光障害葉緑体を除去する選択的オートファジー経路とその誘導機構の解析）

ゲノム継承システム分野

中村 咲耶

Chloroplasts produce energy for plant growth via photosynthesis. As solar energy is converted into chemical energy in chloroplasts for plant growth, chloroplasts also suffer from damage induced by excess energy in sunlight. To maintain effective growth, photodamaged chloroplasts need to be eliminated in an appropriate manner. Plants have developed diverse mechanisms to protect chloroplasts from photodamage. However, how entire damaged chloroplasts are removed has remained poorly understood.

Autophagy is an evolutionally conserved process leading to the degradation of cytoplasmic proteins and organelles in eukaryotes, which facilitates the nutrient recycling and the removal of damaged cellular components. Autophagic delivery processes are mainly categorized into two types: macroautophagy and microautophagy. Macroautophagy is the best characterized type of autophagy, and involves enclosing a portion of the cytosol, including organelles, into a double-membrane-bound vesicle called an autophagosome. The autophagosome fuses with a lysosomal or vacuolar membrane to form an inner-membrane-bound structure for lytic digestion called the autophagic body. *AUTOPHAGY* (*ATG*) genes that are required for the formation of autophagosomal membranes are called “core” *ATGs* (*ATG1-10*, *ATG12-14*, *ATG16*, and *ATG18*) and are essential for all types of macroautophagy. One of these core ATG genes, the ubiquitin-like ATG8 protein, conjugates to a lipid, phosphatidylethanolamine (PE), and forms nascent autophagosomal membranes. ATG3–5, ATG7, ATG10, ATG12 and ATG16 activate this ATG8-PE conjugation pathway.

Direct uptake of cytoplasmic components into the vacuole/lysosomes also occurs by invagination or protrusion of the vacuolar/lysosomal membrane in a process known as microautophagy. However, the molecular mechanism for these processes remains uncertain compared with macroautophagy. In the methylotrophic yeast *Komagataella phaffii* (previously known as *Pichia pastoris*), microautophagy process is characterized as a degradation form of

peroxisomes termed micropexophagy. This process is induced when cell's energy source switches from methanol to glucose. Genetic evidence collected through analyzing the *K. phaffii* mutants suggests that micropexophagy uses the "core" ATG machinery that is essential for macroautophagy. However, in plants, the process of *ATG* gene-dependent microautophagy has not been characterized.

During autophagy, the degradation substrates can be either specifically selected or randomly acquired depending on the circumstances. Heterotrophs produce most of the energy for growth via oxidative phosphorylation within mitochondria during respiration, which results in the production of reactive oxygen species (ROS) and the subsequent accumulation of mitochondrial damage. In yeasts and mammals, dysfunctional mitochondria are eliminated via a selective process of macroautophagy termed mitophagy.

In this thesis, I investigated the involvement of autophagy in the turnover of chloroplasts under photodamage, and found that vacuolar transport of whole chloroplasts through an autophagy process termed chlorophagy is induced by damage acquired from exposure to ultraviolet-B (UVB) in Arabidopsis (*Arabidopsis thaliana*) leaves. This transport did not occur in autophagy-deficient *atg* mutants, which exhibited severe growth inhibition to UVB-induced damage. I also found that chlorophagy is more actively induced by chloroplast damage caused by high visible light (HL), and this phenomenon was accelerated by more severe chloroplast damage due to additional treatment of low temperature or several mutations of previously known repair systems for HL damage. Therefore, this study establishes an autophagy process termed chlorophagy that serves as the elimination of entire photodamaged chloroplasts (Izumi et al., 2017). Then, I further focused on the mechanism of this chlorophagy process, including what types of damage within individual chloroplasts induce chlorophagy, and how chloroplasts, which are much larger than typical autophagosomes, are incorporated into the vacuolar lumen.

I conducted microscopic observations of the intracellular induction of chlorophagy in Arabidopsis leaves and found that mesophyll cells damaged by HL displayed abnormal chloroplasts with a swollen shape and 2.5 times the volume of normal chloroplasts. In wild-type plants, these swollen chloroplasts decreased in agreement with the activation of chlorophagy. In the *atg* mutants, the swollen chloroplasts persisted, and dysfunctional

chloroplasts that had lost chlorophyll fluorescence accumulated in the cytoplasm. Chloroplast swelling and subsequent induction of chlorophagy were suppressed by the application of exogenous mannitol to increase the osmotic pressure outside chloroplasts, or by overexpression of VESICLE INDUCING PROTEIN IN PLASTID 1, which maintains chloroplast envelope integrity. Microscopic observations of autophagy-related membranes showed that swollen chloroplasts are partly surrounded by autophagosomal structures, and are directly engulfed by the tonoplast, as in microautophagy. These results indicate that an elevation in membrane potential inside the chloroplast due to HL-derived envelope damage results in chloroplast swelling as an induction factor for chlorophagy, and that this process mobilizes entire chloroplasts via tonoplast-mediated sequestering to avoid the cytosolic accumulation of dysfunctional chloroplasts (Fig. 1; Nakamura et al., 2018).

I further conducted a forward genetic screening of chlorophagy-deficient mutants in Arabidopsis plants to demonstrate the molecular basis underlying the induction of chlorophagy. I isolated several mutant lines, in which chlorophagy induction is specifically suppressed, from ethyl methanesulfonate-mutagenized seed pool, and found some candidate genes that are required for chlorophagy induction.

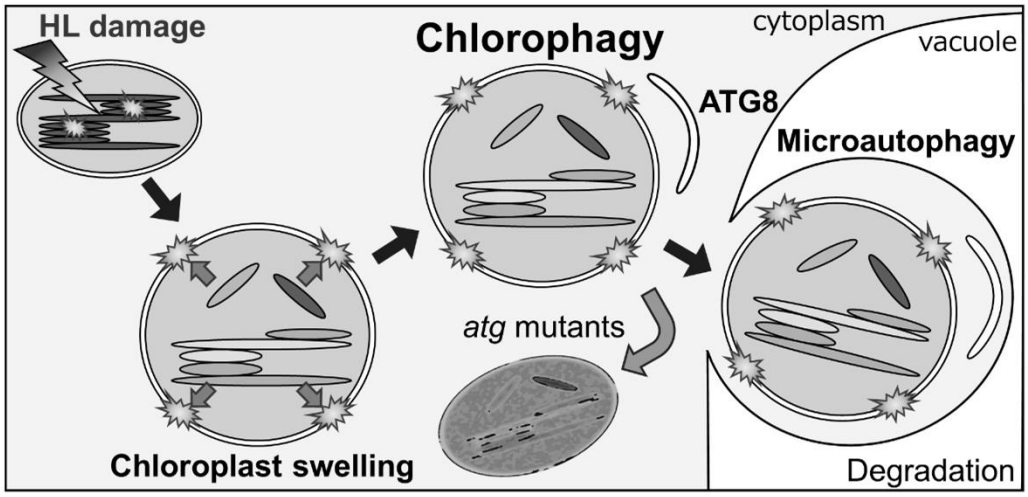

Figure. 1 Schematic model for selective chlorophagy shown in this study.

HL initially causes damage in photosystem II within thylakoid membrane, which then spread to chloroplast envelope. The envelope damage results in an imbalance of transmembrane potential between the stroma and cytoplasm, and manifests as chloroplast swelling. Swollen chloroplasts are marked by autophagosomal structures containing ATG8 and are eliminated into the vacuole by tonoplast-mediated sequestering as a *ATG*-dependent microautophagy process. (Adapted from Nakamura et al., 2018 *Plant Physiol.*)

References

Izumi M, Ishida H, Nakamura S, Hidema J (2017) Entire Photodamaged Chloroplasts Are Transported to the Central Vacuole by Autophagy. Plant Cell **29:** 377-394

Nakamura S, Hidema J, Sakamoto W, Ishida H, Izumi M (2018) Selective elimination of membrane-damaged chloroplasts via microautophagy. Plant Physiol